Water Quality of Lower Deer Creek, Harford County, MD, Home of the Federally Endangered Maryland Darter

Publication No. AFO-C91-01

U.S. Fish and Wildlife Service
Environmental Contaminants Division
Annapolis Field Office
Annapolis, MD 21401

Prepared by:

Elizabeth Block
Environmental Contaminants Biologist

Under supervision of:

Richard O. Bennett, Ph.D., Assistant Supervisor
John P. Wolflin, Supervisor
Annapolis Field Office

October 1990

Title: Water Quality of Lower Deer Creek, Harford County, MD, Home of the Federally Endangered Maryland Darter.

Abstract: The Maryland darter (Etheostoma sellare) is one of the rarest fish in the world, existing in one riffle of Deer Creek, Harford County. There have been several speculations on why the Maryland darter has declined, including loss of habitat from historic submergence of coastal areas or from recent damming, siltation, or overcollection. Regardless of the cause of decline, the Maryland darter population, existing in a single location, is in a precarious situation. This study was a first step in identifying whether contaminants may be threatening the Maryland darter by focusing on one group of contaminants, the organochlorines.

The following samples were collected near the darter riffle and at two sites upstream: sediment, common shiners (Notropis cornutus), Asian clams (Corbicula fluminea) and a variety of benthic invertebrates. To assess water quality, three bioassay organisms were exposed to ambient water: fathead minnow, Ceriodaphnia, and bacteria from the Microtox assay.

Organochlorines were not detected in samples with one exception. Common shiners at the most upstream site contained polychlorinated biphenyls at levels which may be of concern, and contained DDE at levels which are probably not of concern. In general, organochlorine contamination is not a problem in the lower reaches of Deer Creek.

Ambient water collected in May 1989, did not cause significant toxicity to bioassay organisms. However, observations during the summer of 1990 suggested that fish populations may have significantly declined in lower Deer Creek. Deer Creek is designated by the State of Maryland as a scenic river and was believed to have relatively high water quality. If impacts are caused by contaminants, they are likely to be related to pesticide runoff from the primarily agricultural watershed. Water quality-based impacts related to increased sedimentation are also a possibility. Additional studies are currently in progress which will further define the degree and nature of impacts.

Key Words: Maryland darter, common shiner, benthic invertebrates, Corbicula, PCBs, Deer Creek, bioassay, fathead minnow, Ceriodaphnia, Microtox.

ACKNOWLEDGEMENTS

We would like to thank the following people for assistance with collection of samples: G. Andrew Moser, David Sutherland, and Linda Andreasen, currently or formerly of the Annapolis Field Office. Dr. Richard O. Bennett, Ed Pash, and Dolores Orendorf of the Annapolis Field Office provided editorial assistance. Special thanks go to Ron Preston and the staff of the U.S. Environmental Protection Agency Wheeling Laboratory for providing cost-effective bioassays.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

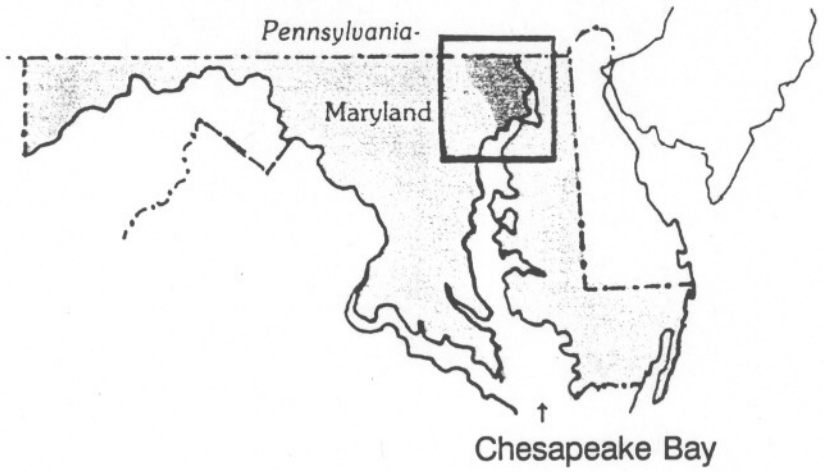
LIST OF APPENDICES

INTRODUCTION

The Maryland darter (Etheostoma sellare) is Federally listed as endangered and is considered to be one of the rarest fish in the world. It is currently known to occur in a single riffle of Deer Creek, Harford County, MD, although specimens have also been collected from one or possibly two nearby creeks (U.S. Fish and Wildlife Service 1985). Even in Deer Creek, its existence is currently questionable, as no the species was last observed there in 1988. Studies are underway to determine its current status (G. Andrew Moser, U.S. Fish and Wildlife Service, Annapolis Field Office, personal communication).

Reasons for the Maryland darter's decline are open for speculation. Deer Creek is a tributary to the Susquehanna River, which is the major freshwater source and the head of the Chesapeake Bay (Figure 1). If appropriate habitat is limited to swift riffles of the Coastal Plain, as is the case for a few other darter species, numbers of Maryland darters could have been substantially reduced during historic submergence of coastal areas which resulted in creation of the Chesapeake Bay. Further reduction may have occurred due to the completion of the Conowingo Dam on the Susquehanna River in 1928, which inundated the mouths of many streams. Anthropogenic increase in sedimentation rates has also been considered a possible explanation for the darter's decline. The original description of the Maryland darter was based on two subadult males taken in 1912 from "Swan Creek near Havre de Grace, Maryland" (Radcliffe and Welsh 1913). Maryland darters have not been located in Swan Creek since then, and considerable sedimentation has occurred. Finally, overcollecting has been cited as a significant impact. The main population of Maryland darters was finally located in 1965 in Deer Creek. At this time, 34 specimens were collected (U.S Fish and Wildlife Service 1985), which may have been a significant portion of the limited population. Regardless of the cause of decline, the Maryland darter population, existing in a single location, is in a precarious situation.

Deer Creek has escaped many of the impacts associated with today's rapid industrial and urban development. Historic industries on Deer Creek included grist mills, sawmills, flint mills, and iron furnaces. Water-powered mills were made obsolete by the development of steam power, and land use in the Deer Creek valley has remained agriculturally oriented up to this day. A 1973 report estimated that approximately 70% of the acreage in the Deer Creek valley was farm land, and most of the remaining 30% was forest or woodlot (Maryland Department of State Planning 1973). No incorporated or unincorporated towns exist along the creek, although communities occur along several tributaries. Development along the creek is limited to a few housing developments, scattered farms, and single family homes (Maryland Department of Natural Resources 1979).



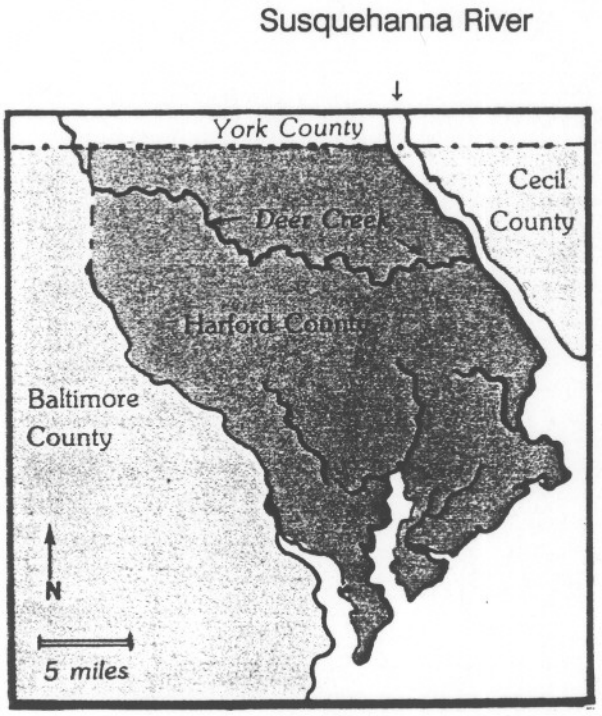


Figure 1. Location of Deer Creek in Harford County, MD. Source: Maryland Department of Natural Resources 1979.

The Maryland Wild and Scenic Rivers Act was passed in 1968, and Deer Creek was designated as a scenic river in 1973. Currently, the State of Maryland classifies Deer Creek and all of its tributaries in use group 3P upstream of the Eden Mill Dam, indicating that the waters are protected for public drinking water supply and naturally occurring trout populations. Downstream of the dam, use classification 1 requires maintenance of water quality for recreational use and support of aquatic life (Don Elmore, Regulations Administrator, Division of Standards and Certification, Water Quality Administration, Maryland Department of Environment, personal communication).

The immediate surroundings of the darter riffle are protected from development, as they are within Susquehanna State Park. However, upstream threats to water quality have occurred. Degradation of water quality occurred in 1985 when one hundred thousand gallons of liquid manure waste accidentally spilled into Deer Creek when a berm gave way. The resultant massive fish kill fortunately did not extend downstream to the Maryland darter's habitat, and subsequent observations confirmed that the species remained extant. The Aberdeen Proving Ground Churchville Test Course for tanks is adjacent to the creek along steeply sloping banks, and may be a source of sedimentation. Four wastewater treatment plants discharge into Deer Creek or its tributaries, and there are four additional permitted industrial discharges including an ordnance testing facility.

The purpose of this study was to determine whether contaminants posed a threat to the limited population of Maryland darters. The land use in the Deer Creek watershed is primarily agricultural, and contaminants of concern were pesticides possibly introduced into Deer Creek as runoff from fields. The study proposal originally included analysis for several classes of pesticides and other contaminants, but was not fully funded, limiting the number of pesticides which could be analyzed for. Samples were analyzed for organochlorines, with the expectation that, as there were no suspected recent sources of these compounds, they could be eliminated from future consideration of possible contaminant problems. Water quality was also assessed through testing of ambient water using three different bioassay organisms.

MATERIALS AND METHODS

Site Description

Three sites on lower Deer Creek, Harford County (Figure 2) were sampled: Stafford Bridge, confluence with Elbow Branch, and Wilson Mill on Route 161. All sites were similar in terms of stream size, flow, substrate, and surrounding vegetation.

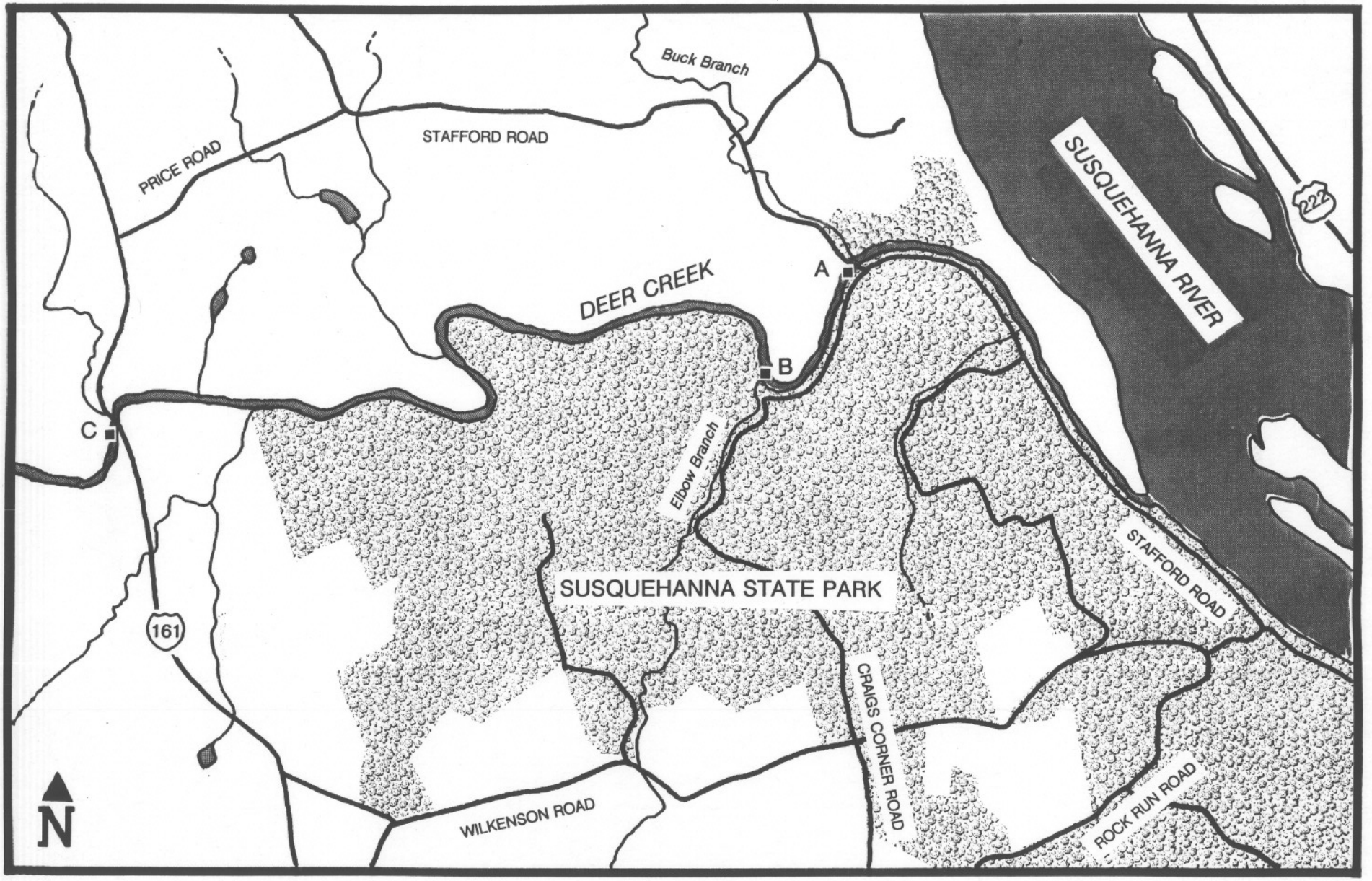


Figure 2. Sampling site locations at Deer Creek, Harford County, MD. Locations are indicated by black boxes: A - Stafford Bridge, B - Elbow Branch Confluence, C - Wilson Mill. Scale is 1 inch = 2000 feet.

At normal low flows, the creek is about 25 meters in width and 1-1½ meters in deep areas. The water moves swiftly over a substrate composed of rubble, rocks, and gravel, with very little siltation. Rooted aquatic plants include riverweed (Podostemum ceratophyllum), Elodea sp., and several species of Potamogeton. The banks adjacent to the stream are sloped and support deciduous forest species typical of the area, including yellow (tulip) poplar (Liriodendron tulipifera), beech (Fagus grandifolia), river birch (Betula nigra), and several species of oaks.

Bioassays

Assessment of water quality included three bioassays performed with the following organisms: the fathead minnow (Pimephales promelas), a cladoceran (Ceriodaphnia dubia), and a bacteria used in the Microtox test (Photobacterium phosphoreum). Three gallons of ambient water was collected from each of the three sites on May 2, 1989, just after a major rainstorm. Water was collected from less turbulent areas of the creek by submerging 1 gallon collapsible plastic containers to overflowing. Water samples were refrigerated until delivery to EPA Wheeling Laboratory, Wheeling, WV, where bioassays were initiated on May 3.

The 7-day fathead minnow and 6-day Ceriodaphnia chronic bioassay test methods conformed to EPA protocols (Horning and Webber 1985). Sample water was treated as follows. Each day, a portion of the samples was removed from the refrigerator and filtered through a 30-micron nylon net into 1600 ml beakers and warmed to test temperature. Diluted mineral water (1 part mineral water to 4 parts deionized water) was used for the control. Routine physical and chemical parameters measured included temperature, pH, conductivity, and dissolved oxygen. Water was then delivered to the appropriate test chambers. At the end of each 24 hour exposure period, dissolved oxygen was again measured in one of the replicates of each fathead minnow test sample. Hardness and alkalinity analyses were performed on two of three ambient samples and each batch of control water. Dissolved oxygen, conductivity, and pH meters were calibrated daily.

Ceriodaphnia were obtained from cultures maintained at Wheeling Laboratory and were from 1 to 4½ hours old at test initiation. Test organisms were transferred from the old test solution to the new solution with an eye dropper. Young were counted and discarded. The number of surviving adults and the number of young produced were recorded daily. The test was terminated on day 6 after production of the third brood.

Fathead minnows were obtained from Wheeling Laboratory cultures and were 23 to 30 hours old at the start of the test. Ten minnows were placed in each of two replicate containers for each sample tested. The number of survivors were recorded daily. At the end of the 7-day test period, fish were euthanized,

temporarily stored in 70% ethanol, and then rinsed, dried with a paper towel, and weighed.

Microtox assays were performed using the Beckman Model 2055 Toxicity Analyzer System with reagents and lyophilized luminescent bacteria supplied by the manufacturer (Beckman Instruments 1982). Samples were adjusted to 2% sodium chloride to support the marine bacteria. The resultant 99% sample concentration was serially diluted (49.5, 24.75, and 12.375%) and run in triplicate, with a 2% sodium chloride diluent blank. Light output was measured at 5 and 15 minutes after adding reconstituted bacteria.

Residue Analysis

Samples of sediment, common shiner (Notropis cornutus), Asian clams (Corbicula fluminea), and a variety of larval aquatic insects were collected from the three sites on July 24 and 31, 1989. Water quality parameters (temperature, pH, dissolved oxygen, and conductivity) were measured with a Hydrolab Surveyor(R) II (Hydrolab Corporation, Austin, TX) on July 24 at Stafford Bridge site only. Sediment was collected at each site from shallow areas along a stretch of shore. The most fine-grained sediment available was scooped into a clean stainless steel mixing bowl, thoroughly mixed, placed in a chemically clean jar (I-Chem Research, New Castle, DE), and placed on ice until it could be frozen.

Fish were collected by kick seining. The seine was set and the substrate within 2 meters upstream was vigorously disturbed to drive fish downstream into the net. We originally intended to collect tesselated darters (Etheostoma olmstedi), but the population was low that year and only one was located. Common shiners were measured for length and weight, wrapped in labelled acetone-washed foil, and placed on ice until they could be frozen. Four individuals were collected at the Stafford Bridge site, and three at the other two sites. Fish samples were analyzed as whole body individuals.

Two types of invertebrate samples were collected, each type at two of the three sites. Benthic invertebrates were collected with a Surber sampler (Wildco Instruments, Saginaw, MI) and composited until a sample weight of 5 grams was obtained. Two such samples were collected at the first site sampled, Stafford Bridge, but this method proved to be too time consuming. A single composite sample was collected at the Elbow Branch site from incidental take in the seine. No invertebrates were collected at the Wilson Mill site. Clams were collected by sieving sandy areas of the stream bed and by visual observation while snorkeling. Soft tissue of six to 27 individual clams was composited until a sample weight of 5 5 grams was obtained. A single clam sample was collected at Stafford Bridge and three samples were collected at Elbow Branch. Clams were not located at Wilson Mill, the furthest upstream site, in spite of diligent search.

Samples were analyzed by Geochemical and Environmental Research Group, Texas A&M University, College Station, TX. Samples were prepared according to National Oceanic and Atmospheric Administration National Status and Trends methods. Sediment samples were lyophilized and extracted with methylene chloride in a Soxhlet extraction apparatus for 12 hours. Extracts were treated with copper to remove sulfur and purified by silica/alumina column chromatography. Tissue samples were simultaneously homogenized and extracted with sodium sulfate and methylene chloride. Extracts were purified by silica/alumina column chromatography to isolate the aliphatic and polycyclic aromatic hydrocarbon (PAH)/pesticide/polychlorinated biphenyl (PCB) fractions. The PAH/pesticide/PCB fraction was further purified by high performance liquid chromatography to remove interfering lipids. Quantitative analysis was performed by capillary gas chromatography equipped with an electron capture detector. Analysis was conducted according to U.S. Fish and Wildlife Service, Patuxent Analytical Control Facility quality control/quality assurance specifications, including analysis of duplicate samples and assessment of percent recovery.

RESULTS

Water quality parameters measured during bioassay testing and during sample collection are presented in Table 1. The values do not indicate water quality problems, with the possible exception of a somewhat low pH reading during sample collection. Lower dissolved oxygen values measured during bioassay testing were most likely a result of water sample treatment during testing.

Bioassay results (Table 2) indicated no adverse impacts to test organisms from ambient Deer Creek water. Ceriodaphnia adults maintained 100% survival, and the mean number of young produced in Deer Creek water was greater than in control water. Fathead minnows showed some decreased survival and lower growth weight compared to controls, but neither percent survival nor mean dry weight was significantly lower than controls according to standard bioassay statistical tests. Microtox bacteria showed small reductions in luminescence, but these reductions did not indicate toxicity. Detailed bioassay results appear in Appendix 1.

The compounds analyzed for in fish, invertebrate, and sediment samples are listed in Table 3. Of all samples, only three common shiners contained detectable levels of these compounds. Levels are reported as parts per million (ppm) wet weight. A single common shiner from the Stafford Bridge site contained 0.05 ppm of p,p'-DDE. Two shiners from Wilson Mill contained detectable levels of contaminants: one contained 0.52 ppm total PCBs, and the other contained 0.09 ppm p,p'-DDE and 0.82 ppm total PCBs.

Table 1. Physical conditions of water collected for bioassays and during residue sample collection from lower Deer Creek, Harford County, MD.

Sampling Site	Dissolved O_2 (mg/L)	pH	Temperature (°C)	Conductivity (μmhos/cm)	Alkalinity (mg/L)	Hardness (mg/L)
Stafford Bridge, bioassay	4.0 - 10.6	6.3 - 6.6	23.5 - 25.0	115 - 120	*	*
Elbow Branch Confluence	4.0 - 11.0	6.2 - 6.4	23.5 - 25.0	115 - 120	21.0	32.0
Wilson Mill	4.1 - 11.6	6.2 - 6.5	23.5 - 25.0	115 - 118	19.0	36.0
Laboratory Control	5.0 - 8.2	7.3 - 7.5	23.0 - 25.0	170 - 180	60.0	82.0
Stafford Bridge, residue sample collection	9.2	5.8	24	130	**	**

* No results due to insufficient sample size
** Not determined

Table 2. Results from three bioassay organisms exposed to water from lower Deer Creek, Harford County, MD.

	CERIODAPHNIA DUBIA		FATHEAD MINNOW		MICROTOX
Sampling Site	Percent Adult Survival	Mean Young Produced	Percent Survival	Mean Dry Weight (mg)	Percent Light Reduction
Stafford Bridge	100	37.80	80	0.50	1.7
Elbow Branch Confluence	100	37.20	95	0.45	0.0
Wilson's Mill	100	38.30	85	0.45	3.0
Laboratory Control	100	34.10	100	0.52	not tested

Table 3. Organochlorine compounds analyzed for in fish, invertebrates, and sediment from lower Deer Creek, Harford County, MD.

Alpha-benzene hexachloride (BHC)
Beta-BHC
Gamma-BHC
Delta-BHC
Total BHCs

Heptachlor
Heptachlor Epoxide

Alpha-chlordane
Gamma-chlordane
Oxychlordane
Cis-nonachlor
Trans-nonachlor

O,P' DDT
P,P' DDT
O,P' DDD
P,P' DDD
O,P' DDE
P,P' DDE

Aldrin
Dieldrin
Endrin
Lindane (BHC)
Mirex
Toxaphene

Total Polychlorinated Biphenyls (PCB)

DISCUSSION

Results from bioassays and measurement of physical parameters indicate that Deer Creek did not suffer from levels of pollution which would cause toxicity or grossly affect water quality, at least during the times the study was conducted. Chemical analysis of biotic and abiotic samples indicated that, overall, lower Deer Creek is not impacted by organochlorines.

Of the compounds found in common shiners from Wilson Mill, detected levels of DDE appear not to be a problem. Levels were far below the 5.0 ppm criteria level established by the U.S. Food and Drug Administration for the protection of human health through consumption of fish (U.S. Food and Drug Administration 1980), and well below the 1.0 ppm criteria level for the protection of fish-eating wildlife established by the National Academy of Sciences and National Academy of Engineers (NAS/NAE 1972). Also, DDE is the final metabolite product of DDT and DDD. Lack of detection of DDT and DDD may indicate that Deer Creek is not receiving recent inputs of this highly persistent compound.

PCB levels detected in common shiners may indicate some impact to biota in the Wilson Mill vicinity. Two of three fish contained levels above the NAS/NAE criteria level of 0.5 ppm. Whole body residues of 0.4 ppm PCBs have been associated with reproductive toxicity in rainbow trout (U.S. Environmental Protection Agency 1980). Further examination of PCB contamination at Wilson Mill and upstream portions of Deer Creek may be warranted.

Overall, this study was successful in concluding that organochlorine compounds are not impacting the Maryland Darter and other biota in lower Deer Creek. However, other anthropogenic impacts cannot be ruled out. In fact, a series of recent observations have indicated that Deer Creek fish populations are being significantly impacted.

Dr. Rich Raesly has snorkeled the Maryland darter riffle to determine darter population levels over several years. This year, he noticed that numbers of fish of all species were not nearly as abundant as in previous years. In light of this observation, he seined a stretch of Deer Creek at the Route 161 crossing which he had also seined in 1986. Both the number of species and number of individuals had decreased dramatically. (Dr. Rich Raesly, Frostburg State University, Department of Biology, personal communication).

Five species of darters in addition to the Maryland darter are found in the lower reaches of Deer Creek, of which the tesselated darter, shield darter (Percina peltata), and introduced banded darter (Eatheostoma zonale) were previously abundant. Dr. Raesly reported that, typically, 20-30 darters were observed per

hour during snorkeling. Less than one per hour was observed by Dr. Raesly during snorkeling in summer 1990. No tesselated darters were taken in the seine during collection of samples for contaminant analysis.

This decline in fish populations is cause for concern, particularly as Deer Creek has always been considered to have relatively high water quality. The cause for the decline is not immediately apparent. Two upcoming studies will address possible impacts. Water samples were collected at four sites in Deer Creek by U.S. Fish and Wildlife Service Annapolis Field Office staff in July 1990, and will be analyzed for organophosphate and carbamate insecticides and herbicides. A study conducted by Dr. Raesly will sample 10 stations throughout the Maryland portion of Deer Creek in late summer and fall. Fish populations will be assessed by seining and electroshocking, and water samples will be analyzed for water quality parameters including organic acids, nutrients, and dissolved and suspended solids.

Deer Creek is special in that it is relatively unimpacted by industry and urbanization from headwaters to confluence. We recommend that study results be reviewed and further studies be conducted as necessary to define the cause and extent of reduction in fish populations.

LITERATURE CITED

Beckman Instruments. 1982. Microtox system operating manual. Beckman Instruments, Carlsbad, CA.

Horning, W. III, and C. Webber. 1985. Short-term methods for estimating the chronic toxicity of effluents and receiving waters to freshwater organisms. U.S. Environmental Protection Agency Report No. EPA/600-4-85-014, Cincinnati, OH.

Maryland Department of Natural Resources. 1979. Deer Creek Scenic River, revised edition. Department of General Services purchase order # 434-01. Maryland Department of Natural Resources, Annapolis, MD.

Maryland Department of State Planning. 1973. MAGI System, 1973 land use map of Maryland.

National Academy of Sciences and National Academy of Engineering. 1972. Section III - Freshwater aquatic life and wildlife, water quality criteria. Ecological Research Series, EPA-R3-033, March 1973:106-113.

Radcliffe, L. and W. W. Welsh. 1913. Description of a new darter from Maryland. Bull. U.S. Bur. Fish 32:29-32.

U.S. Environmental Protection Agency. 1980. Ambient water quality criteria for polychlorinated biphenyls. U.S. EPA Rep. 440/5-80-068. 211pp.

U.S. Fish and Wildlife Service. 1985. The Maryland darter recovery plan. USFWS Region 5, Newton Corner, MA. 38pp.

U.S. Food and Drug Administration. 1980. Action levels for poisonous or deleterious substances in human food and animal feed. Pub. No. HFF-326. FDA, Washington, D.C. 13pp.

APPENDIX 1. Bioassay results.

Toxicity Test Report

MARYLAND DARTER STUDY

U.S. Fish & Wildlife Service
Annapolis, MD

6/21/89

Wheeling Office Biology Group
James Green
Robert Donaghy
Lynne Bailey

U.S. Environmental Protection Agency
Wheeling Operations Section
303 Methodist Building, 11th & Chapline Streets
Wheeling, West Virginia 26003

INTRODUCTION

On May 2, 1989, surface water samples were collected at three locations in Harford County, Maryland as part of the U.S. Fish & Wildlife Maryland Darter Study. These samples were delivered to the Wheeling Laboratory and stored in the refrigerator on May 3, 1989. Ceriodaphnia dubia chronic toxicity tests were set up on May 3 and terminated on May 9 after all the test organisms had produced three broods. The fathead minnow (Pimephales promelas) tests were set up on May 3 and terminated on May 10. A Microtox toxicity test was performed on May 4, 1989.

CONCLUSIONS

These ambient samples had no adverse impact on the Ceriodaphnia dubia or the fathead minnows. Some mortalities were observed during the fathead minnow test; however, they were not significant. There was no significant difference among the results of the fathead minnow growth results. The Microtox bacteria were also not adversely affected. These results indicate that chronic toxicity to the aquatic community is not effective in the stream samples collected for this study.

TEST METHODS

General

The fathead and Ceriodaphnia test methods conformed in general with the "Short-Term Methods for Estimating the

procedure was repeated using a second brood for replicates 5-7, and a third brood for replicates 8-10. Each cup contained 20 ml of test solution, 100 ul of a trout chow-yeast-alfalfa mixture and 200 ul of a concentrated Selenastrum capricornutum culture. The daily renewals were accomplished by dispensing fresh food and new test solutions to new cups. The test organisms were transferred from the old test solution to the new solution with an eye dropper. When young were present, they were counted and discarded. The number of surviving adults and the number of young produced were recorded daily. The test was terminated on day six after all animals had produced their third brood.

Fathead Minnow Test Methods

The fathead minnows used for these tests were obtained from the Wheeling laboratory cultures. They were 23 - 30 hours old at the start of the tests. Crystallizing dishes, 125 mm diameter by 65 mm deep, were used for test containers. They were washed with soap and hot water and rinsed with tapwater, then rinsed with 15% hydrochloric acid and tapwater, and finally rinsed with acetone and tapwater prior to use. The tests were initiated by placing 400 ml of test solution in each of two replicate containers for each sample tested. Ten larval fathead minnows were placed in each test container using a wide-bore pipette and containers were randomly positioned on a laboratory cart. They were fed 0.1 ml of

Serial dilutions of 49.5%, 24.75% and 12.375% were prepared with a 2% sodium chloride diluent reagent and triplicate 1 ml aliquots of each of the four concentrations as well as a 2% sodium chloride diluent blank provided by the manufacturer were dispensed to cuvettes.

Sample and control aliquots were precooled in incubator wells to 15 degrees centigrade at which time 10 ul of the reconstituted test organisms were added to each aliquot. After a five and fifteen minute exposure period, light output measurements were made utilizing the photometer portion of the analyzer system and recorded on the attached strip recorder. These data were reduced by calculating the mean percent difference between the control light output and that of the sample.

RESULTS

General

Summaries of the Ceriodaphnia dubia, fathead minnow and Microtox results are located in Table 1. The daily Ceriodaphnia survival percentages can be found in Table 2 and the daily mean number of young produced can be found in Table 3. The fathead minnow daily survival results are located in Table 4. A summary of the chemical and physical data obtained during the tests is presented in Table 5. Appendix A contains the

Toxicity Test Report

MARYLAND DARTER STUDY

U.S. Fish & Wildlife Service
Annapolis, MD

6/21/89

Wheeling Office Biology Group
James Green
Robert Donaghy
Lynne Bailey

U.S. Environmental Protection Agency
Wheeling Operations Section
303 Methodist Building, 11th & Chapline Streets
Wheeling, West Virginia 26003

INTRODUCTION

On May 2, 1989, surface water samples were collected at three locations in Harford County, Maryland as part of the U.S. Fish & Wildlife Maryland Darter Study. These samples were delivered to the Wheeling Laboratory and stored in the refrigerator on May 3, 1989. Ceriodaphnia dubia chronic toxicity tests were set up on May 3 and terminated on May 9 after all the test organisms had produced three broods. The fathead minnow (Pimephales promelas) tests were set up on May 3 and terminated on May 10. A Microtox toxicity test was performed on May 4, 1989.

CONCLUSIONS

These ambient samples had no adverse impact on the Ceriodaphnia dubia or the fathead minnows. Some mortalities were observed during the fathead minnow test; however, they were not significant. There was no significant difference among the results of the fathead minnow growth results. The Microtox bacteria were also not adversely affected. These results indicate that chronic toxicity to the aquatic community is not effective in the stream samples collected for this study.

TEST METHODS

General

The fathead and Ceriodaphnia test methods conformed in general with the "Short-Term Methods for Estimating the

procedure was repeated using a second brood for replicates 5-7, and a third brood for replicates 8-10. Each cup contained 20 ml of test solution, 100 ul of a trout chow-yeast-alfalfa mixture and 200 ul of a concentrated Selenastrum capricornutum culture. The daily renewals were accomplished by dispensing fresh food and new test solutions to new cups. The test organisms were transferred from the old test solution to the new solution with an eye dropper. When young were present, they were counted and discarded. The number of surviving adults and the number of young produced were recorded daily. The test was terminated on day six after all animals had produced their third brood.

Fathead Minnow Test Methods

The fathead minnows used for these tests were obtained from the Wheeling laboratory cultures. They were 23 - 30 hours old at the start of the tests. Crystallizing dishes, 125 mm diameter by 65 mm deep, were used for test containers. They were washed with soap and hot water and rinsed with tapwater, then rinsed with 15% hydrochloric acid and tapwater, and finally rinsed with acetone and tapwater prior to use. The tests were initiated by placing 400 ml of test solution in each of two replicate containers for each sample tested. Ten larval fathead minnows were placed in each test container using a wide-bore pipette and containers were randomly positioned on a laboratory cart. They were fed 0.1 ml of

Serial dilutions of 49.5%, 24.75% and 12.375% were prepared with a 2% sodium chloride diluent reagent and triplicate 1 ml aliquots of each of the four concentrations as well as a 2% sodium chloride diluent blank provided by the manufacturer were dispensed to cuvettes.

Sample and control aliquots were precooled in incubator wells to 15 degrees centigrade at which time 10 ul of the reconstituted test organisms were added to each aliquot. After a five and fifteen minute exposure period, light output measurements were made utilizing the photometer portion of the analyzer system and recorded on the attached strip recorder. These data were reduced by calculating the mean percent difference between the control light output and that of the sample.

RESULTS

General

Summaries of the Ceriodaphnia dubia, fathead minnow and Microtox results are located in Table 1. The daily Ceriodaphnia survival percentages can be found in Table 2 and the daily mean number of young produced can be found in Table 3. The fathead minnow daily survival results are located in Table 4. A summary of the chemical and physical data obtained during the tests is presented in Table 5. Appendix A contains the

Table 1
MD Darter Study
Data Summary

Station		Ceriodaphnia dubia Percent Adult Survival	Ceriodaphnia dubia Mean Young Produced	Fathead Minnow Percent Survival	Fathead Minnow Mean Dry Weight (mg)
MD Darter Study -	Site A	100	37.80	80	0.50
	Site B	100	37.20	95	0.45
	Site C	100	38.30	85	0.45
	Control	100	34.10	100	0.52

Microtox Test Results

Station	Conc. Tested	Test Results
MD Darter Study Site A	99%	Percent light reduction after
	49.5%	15 minutes exposure = 1.7%
	24.75%	
	12.375%	
	0%	
MD Darter Study Site B	99%	Percent light reduction after
	49.5%	15 minutes exposure = 0%
	24.75%	
	12.375%	
	0%	
MD Darter Study Site C	99%	Percent light reduction after
	49.5%	15 minute exposure = 3%
	24.75%	
	12.375%	
	0%	

MD Darter Study

Table 2
Ceriodaphnia dubia % survival

Station		Day 1	2	3	4	5	6
MD Darter Study -	Site A	100	100	100	100	100	100
	Site B	100	100	100	100	100	100
	Site C	100	100	100	100	100	100
	Control	100	100	100	100	100	100

Table 3
Mean Young Ignoring Mortality

Station		Day 3	4	5	6	Total
MD Darter Study -	Site A	5.60	0.50	12.00	19.70	37.80
	Site B	5.40	0.00	12.10	19.70	37.20
	Site C	5.90	0.00	13.60	18.80	38.30
	Control	5.70	0.00	10.80	17.60	34.10

MD Darter Study

Table 4
Fathead Minnow % Survival

Station	Day 1	2	3	4	5	6	7
MD Darter Study - Site A	90	90	85	85	85	85	80
Site B	100	100	95	95	95	95	95
Site C	100	100	100	100	100	100	85
Control	100	100	100	100	100	100	100

MD Darter Study

Table 5
Physical/Chemical Data

Concentration	D.O. (mg/l)	pH	Temp. (C)	Cond. (umhos/cm)	Alk. (mg/l)	Hardness (mg/l)
Site A	4.0-10.6	6.3-6.6	23.5-25.0	115-120	*	*
Site B	4.0-11.0	6.2-6.4	23.5-25.0	115-120	21.0	32.0
Site C	4.1-11.6	6.2-6.5	23.5-25.0	115-118	19.0	36.0
Control	5.0-8.2	7.3-7.5	23.0-25.0	170-180	60.0	80.0-84.0

* No results due to insufficient sample

APPENDIX A

Number of Ceriodaphnia young produced by each adult during each interval between observations.

Sample description: MD Darter Study - Site A
Test date: 5/3-9/89

Interval	Replicate A	B	C	D	E	F	G	H	I	J	Living Females	MIM*
0-72 hr.	6	6	7	7	7	7	6	5	5	0	10.0	5.60
72-96 hr.	0	0	0	0	0	0	0	0	0	5	10.0	0.50
96-120 hr.	13	10	11	13	15	14	12	10	12	10	10.0	12.00
120-144 hr.	18	20	19	21	21	19	23	17	20	19	10.0	19.70
											Percent Survival	
Total	37	36	37	41	43	40	41	32	37	34	100	37.80

Sample description: MD Darter Study - Site B
Test date: 5/3-9/89

Interval	Replicate A	B	C	D	E	F	G	H	I	J	Living Females	MIM*
0-72 hr.	6	7	6	7	5	7	3	2	7	4	10.0	5.40
72-96 hr.	0	0	0	0	0	0	0	0	0	0	10.0	0.00
96-120 hr.	14	12	12	14	14	12	6	10	13	14	10.0	12.10
120-144 hr.	20	24	22	24	20	16	16	17	17	21	10.0	19.70
											Percent Survival	
Total	40	43	40	45	39	35	25	29	37	39	100	37.20

Sample description: MD Darter Study - Site C
Test date: 5/3-9/89

Interval	Replicate A	B	C	D	E	F	G	H	I	J	Living Females	MIM*
0-72 hr.	8	6	8	5	5	7	6	4	5	5	10.0	5.90
72-96 hr.	0	0	0	0	0	0	0	0	0	0	10.0	0.00
96-120 hr.	15	16	13	14	14	14	15	10	11	14	10.0	13.60
120-144 hr.	20	19	22	20	16	23	17	14	18	19	10.0	18.80
											Percent Survival	
Total	43	41	43	39	35	44	38	28	34	38	100	38.30

---

*MIM - Mean young Ignoring Mortality
D - Adult died during interval
- No counts made

APPENDIX A

Number of Ceriodaphnia young produced by each adult during each interval between observations.

Sample description: MD Darter Study - Control
Test date: 5/3-9/89

Interval	Replicate A	B	C	D	E	F	G	H	I	J	Living Females	MIM*
0-72 hr.	6	5	5	7	6	5	7	7	3	6	10.0	5.70
72-96 hr.	0	0	0	0	0	0	0	0	0	0	10.0	0.00
96-120 hr.	10	12	11	11	12	11	12	12	5	12	10.0	10.80
120-144 hr.	17	17	16	19	19	17	18	18	13	22	10.0	17.60
											Percent Survival	
Total	33	34	32	37	37	33	37	37	21	40	100	34.10

*MIM - Mean young Ignoring Mortality
D - Adult died during interval
- No counts made

APPENDIX B

Fathead Minnow, Chronic Test Data

Sample Descrip.	Rep.	No. Fish	Pan Wt.	Total Wt.	Net Wt.	Mean Wt.	% Sur.
MD Darter Study Site A	A	8	1422.79	1426.58	3.79	0.47	80
	B	8	989.83	993.97	4.14	0.52	80
				Overall Mean Wt.		0.50	
				Standard Deviation		0.02	
				Coe. of Var.		4.41	
MD Darter Study Site B	A	9	1409.03	1413.19	4.16	0.46	90
	B	10	1400.00	1404.29	4.29	0.43	100
				Overall Mean Wt.		0.45	
				Standard Deviation		0.02	
				Coe. of Var.		3.73	
MD Darter Study Site C	A	10	1402.65	1407.29	4.64	0.46	100
	B	7	1399.10	1402.12	3.02	0.43	70
				Overall Mean Wt.		0.45	
				Standard Deviation		0.02	
				Coe. of Var.		3.64	
MD Darter Study Control	A	10	1397.92	1403.34	5.42	0.54	100
	B	10	1399.88	1404.95	5.07	0.51	100
				Overall Mean Wt.		0.52	
				Standard Deviation		0.02	
				Coe. of Var.		3.34	

All weights in milligrams